

1265807

UNITED STATES PATENT AND TRADEMARK OFFICE

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

*December 23, 2004*

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM  
THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK  
OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT  
APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A  
FILING DATE.

APPLICATION NUMBER: 60/518,988

FILING DATE: *November 10, 2003*

RELATED PCT APPLICATION NUMBER: PCT/US04/38252

Certified by



Jon W Dudas

Acting Under Secretary of Commerce  
for Intellectual Property  
and Acting Director of the U.S.  
Patent and Trademark Office



**BEST AVAILABLE COPY**

Mail Stop Provisional Patent Application  
Assistant Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

PTO/SB/16 (6/95) (Modified)  
EXPRESS MAIL NO.EV298564123US

## PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 C.F.R. 1.53 (c)

02570 U.S. PTO

Docket Number	1339.026PRV	Type a plus sign (+) inside this box >	+
Customer No.	21186	Confirmation No.	

INVENTOR(s)/APPLICANT(s)					
Name (last, first, middle initial)			RESIDENCE (CITY, AND EITHER STATE OR FOREIGN COUNTRY)		
Krasutsky, Pavel A. Kolomitsyna, Oksana Krasutskyy, Dmytro A. Kacharov, Olexiy D.			Duluth, MN Duluth, MN Duluth, MN Duluth, MN		
TITLE OF THE INVENTION (280 characters max)					
BIRCH BARK EXTRACT PROCESSING UTILIZING AZEOTROPIC DISTILLATION					
CORRESPONDENCE ADDRESS					
Schwegman, Lundberg, Woessner & Kluth P. O. Box 2938 Minneapolis, Minnesota 55402					
Attn: Gary J. Speier					
STATE	Minnesota	ZIP CODE	55402	COUNTRY	United States of America
ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/>	Specification	Number of Pages	29	Small Entity Statement	
<input checked="" type="checkbox"/>	Drawing(s)	Number of Sheets	2	Other (specify)	
METHOD OF PAYMENT (check one)					
<input checked="" type="checkbox"/>	A check or money order is enclosed to cover the Provisional filing fees			PROVISIONAL FILING FEE AMOUNT	\$160.00
<input checked="" type="checkbox"/>	The Commissioner is hereby authorized to charge any additional required fees or credit overpayment to Deposit Account Number: <u>19-0743</u>				

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

No.

Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

SIGNATURE 

Date November 10, 2003

TYPED OR PRINTED NAME Gary J. Speier

REGISTRATION NO. 45,458

Additional inventors are being named on separately numbered sheets attached hereto.

**PROVISIONAL APPLICATION FILING ONLY**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re PROVISIONAL Patent Application of: Pavel A. Krasutsky et al.

Title: BIRCH BARK EXTRACT PROCESSING UTILIZING AZEOTROPIC DISTILLATION

Docket No.: 1339.026PRV

**MAIL STOP PROVISIONAL APPLICATION**

Assistant Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313-1450

We are transmitting herewith the following attached items (as indicated with an "X"):

- A PROVISIONAL Patent Application comprising:
  - Specification (29 pgs, including claims numbered 1 through 67 and a 1 page Abstract )
  - 2 Sheet(s) of drawing(s).
    - Signed Combined Declaration and Power of Attorney ( pgs).
  - A check in the amount of \$160.00 to cover the Provisional Filing Fee.
- Provisional Application Cover Sheet (1 page).
- An executed Assignment to ( pages), recordation cover sheet (1 page), and a check for the recordation fee of \$40.00.
- A verified statement to establish small entity status under 37 C.F.R. 1.9 and 1.27 (1 page).
- A return postcard.

**Please charge any additional required fees or credit overpayment to Deposit Account No. 19-0743.**

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.

Customer Number: 21186

By: 

Gary J. Speier  
Reg. No. 45,458

"Express Mail" mailing label number: EV298564123US

Date of Deposit: November 10, 2003

This paper or fee is being deposited on the date indicated above with the United States Postal Service pursuant to 37 CFR 1.10, and is addressed to the Assistant Commissioner for Patents, Attn: MAIL STOP PROVISIONAL PATENT APPLICATION, P.O. Box 1450, Alexandria, VA 22313-1450.

(NEW FILING)

**BIRCH BARK EXTRACT PROCESSING  
UTILIZING AZEOTROPIC DISTILLATION**

5

**Background of the Invention**

Birch bark is a low-value waste product in the forest industry today. Ekman, R., Holzforschung, (1983) 37, 205. Approximately 230,000 tons of birch bark are generated per year. For example, a single paper mill can generate 70 tons of birch bark per day. Thus, vast quantities of birch bark and its chemical components are available.

10 Birch bark is a potential source of a variety of organic chemicals. Several triterpenoids have been identified in birch bark extracts. For example, lupeol, betulin, betulinic aldehyde, betulinic acid, methyl betulinate, lupenone, betulonic aldehyde, betulonic acid,  $\beta$ -amyrin, erythrodiol, oleanolic aldehyde, oleanolic acid, methyl leanolate and acetyl oleanolic acid are all present in the outer bark of *Betula verrucosa*. Eckerman, C., (1985) Paperi ja Puu, No. 3, 100. In addition, several suberic acids isolated from 15 birch bark, as well as several triterpenoids, have been identified in the bark of *Betula verrucosa*. Ekman, R., Holzforschung, (1983) 37, 205.

The chemical constituents of birch bark are useful in pharmaceutical and industrial applications. For example, U.S. Pat. No. 5,750,578 discloses that betulin 20 possesses antiviral properties and is useful to treat herpes virus. Betulin also possesses anti-feedant activity against boll weevils, and anti-inflammatory activity (Miles, D. H., 1994, *J. Agric. Food. Chem.*, 42, 1561-1562 and Recio, M., *Planta Med.*, 1995, 61, 9-12. In addition, betulin showed cough suppressant and expectorant effects. Jinuhua, W., Zhongguo Yaoxue Zazhi, (1994), 29(5), 268-71. Betulin is also a useful starting material 25 for preparing alobetulin and derivatives thereof, which posses useful pharmacological properties.

Betulin can be converted to betulinic acid, which is useful as a therapeutic agent. For example, Pisha, E. et al., (1995) *J. M. Nature Medicine*, 1, 1046-1051 discloses that betulinic acid has anti-tumor activity against human melanoma, e.g., MEL-1, MEL-2 and 30 MEL-4. In addition, Fujioka, T. et al., *J. Nat. Prod.*, (1994) 57, 243-247 discloses that betulinic acid has anti-HIV activity in H9 lymphocytic cells.

Current methods for isolating the chemical constituents of birch bark are deficient in several ways. For example, betulin has been extracted from the bark of white-barked birches in amounts up to 30%, based on the dry weight of the bark. Elkman, R., (1983) Holzforsch, 37, 205; Ohara, S., et al., (1986) Mokuza Gakkaishi, 32, 266. In addition, 5 betulin has been isolated from outer birch bark waste of *Betula verrucosa* by liquid extraction employing boiling organic solvents and subsequent recrystallization. Eckerman, C., (1985) Paperi ja Puu, No. 3, 100. While current processes afford acceptable yields of betulin (e.g., 11-30%), these processes suffer from several major drawbacks. Specifically, the current methods employed to isolate not only betulin, but 10 other components in birch bark (e.g., lupeol and betulinic acid) are costly, inefficient and/or are unsafe.

Russian Patent Nos. RU2175326 (publication date 27 October 2001) and RU2192879 (publication date 20 November 2002), discloses methods of preparing betulin, and derivatives thereof. The methods disclosed in Russian Patent No. 15 RU2192879 include birch bark milling, separation of birch bark fibers, extraction of birch bark, separation of solution from extracted birch bark, and solvent removal from solution. The birch bark extraction is carried out with toluene at temperature of 90°C-110°C for 1.5-3.0 h, and the solution is filtered at a temperature of 40°C-50°C. The toluene betulin solution is cooled for 6-10 h to a temperature of 15°C - 5°C.

20 Published U.S. Patent Application US 2003/0153776 A1 ("the '776 patent application), assigned to Boehringer Ingelheim Pharma, discloses a process for obtaining betulin from birch bark (see Abstract). The process includes extracting birch bark with a high-boiling, water-immiscible solvent, and extracting this extract with a dilute aqueous base (see claim 1).

25 This process is unsuitable for the industrial scale recovery of betulin, as well as lupeol and betulinic acid. The yields and purities of betulin disclosed in the '776 patent application are believed to be erroneous, utilizing the processes described therein. Even if accurate, the yields and purities of betulin disclosed in the '776 patent application can be improved. The processes described therein cannot be practiced on larger, industrial 30 scales (e.g., kilograms or tons).

A need therefore exists for safer, more cost-efficient methods to obtain commercial quantities (e.g., tons) of betulin; as well as commercial quantities (e.g., kg) of lupeol and betulinic acid from birch bark.

5

### Summary of the Invention

The present invention provides a method for obtaining a natural product from plant material. The method includes: (a) contacting plant material with a solvent to provide a first mixture; (b) separating the plant material from the solvent to provide a first extract; (c) contacting the first extract with an aqueous base to provide a second mixture; 10 (d) heating the second mixture in a water-immiscible solvent, effective to distill off water present in the second mixture, thereby providing a third mixture; (e) separating solids from the third mixture to provide a fourth mixture; and (f) concentrating the fourth mixture, or precipitating solids from the fourth mixture, to provide a natural product.

The present invention also provides a method for obtaining betulin from birch bark. The method includes: (a) contacting birch bark with a solvent to provide a first mixture; (b) separating the birch bark from the solvent to provide a first extract; (c) 15 contacting the first extract with an aqueous base to provide a second mixture; (d) heating the second mixture in a water-immiscible solvent, effective to distill off water present in the second mixture, thereby providing a third mixture; (e) separating solids from the third mixture to provide a fourth mixture; and (f) concentrating the fourth mixture to provide 20 betulin, or precipitating betulin from the fourth mixture.

The present invention also provides a method for obtaining lupeol from birch bark. The method includes: (a) contacting birch bark with a solvent to provide a first mixture; (b) separating the birch bark from the solvent to provide a first extract; (c) 25 contacting the first extract with an aqueous base to provide a second mixture; (d) heating the second mixture in a water-immiscible solvent, effective to distill off water present in the second mixture, thereby providing a third mixture; (e) separating solids from the third mixture to provide a fourth mixture; (f) precipitating solids from the fourth mixture; (g) separating the solids from the fourth mixture to provide a mother liquor; and (h) 30 concentrating the mother liquor to provide lupeol; (i) optionally washing the lupeol with a polar organic solvent; and (j) optionally drying the lupeol.

The present invention also provides a method for obtaining betulinic acid from birch bark. The method includes: (a) contacting birch bark with a solvent to provide a first mixture; (b) separating the birch bark from the solvent to provide a first extract; (c) contacting the first extract with an aqueous base to provide a second mixture; (d) heating 5 the second mixture in a water-immiscible solvent, effective to distill off water present in the second mixture, thereby providing a third mixture; (e) separating solids from the third mixture; (f) neutralizing or acidifying the solids in an aqueous acid, thereby providing a fourth mixture; (g) separating betulinic acid from the fourth mixture; (h) optionally washing the betulinic acid with a polar organic solvent; and (i) optionally drying the 10 betulinic acid.

The present invention also provides a method for purifying an extract of a natural product. The method includes: (i) contacting an extract of a natural product with an aqueous base, to provide a first mixture; (ii) heating the first mixture in a water- 15 immiscible solvent, effective to distill off water present in the first mixture, thereby providing a second mixture; (iii) separating solids from the second mixture, to provide a third mixture; and (iv) concentrating the third mixture or precipitating solids from the third mixture, to provide a purified natural product.

The contacting of the extract with an aqueous base and heating in a water- immiscible solvent, effective to distill off water present, can specifically include 20 azeotropic distillation.

The present invention also provides a compound obtained from the method of the present invention.

The present invention also provides a pharmaceutical composition that includes a pharmaceutically acceptable carrier and a compound obtained from the method of the 25 present invention.

The present invention also provides a cosmetic composition that includes a cosmetically acceptable carrier and a compound obtained from the method of the present invention.

**Brief Description of the Figures**

Embodiments of the invention may be best understood by referring to the following description and accompanying drawings which illustrate such embodiments.

The numbering scheme for the Figures included herein are such that the leading number

5 for a given reference number in a Figure is associated with the number of the Figure.

Reference numbers are the same for those elements that are the same across different Figures. For example, a block flow diagram depicting outer birch bark (2) can be located in Figure 1. However, reference numbers are the same for those elements that are the same across different Figures. In the drawings:

10 **Figure 1** illustrates a block flow diagram depicting the isolation of betulin, lupeopl, and/or betulinic acid from birch bark, employing azeotropic distillation.

**Figure 2** illustrates a block flow diagram depicting the isolation of acidic components (natural products) and non-acidic components (natural products) from plant material, employing azeotropic distillation.

15

**Detailed Description of the Invention**

Specific values listed below for ranges are for illustration only; they do not exclude other defined values or other values within defined ranges.

20 **Definitions**

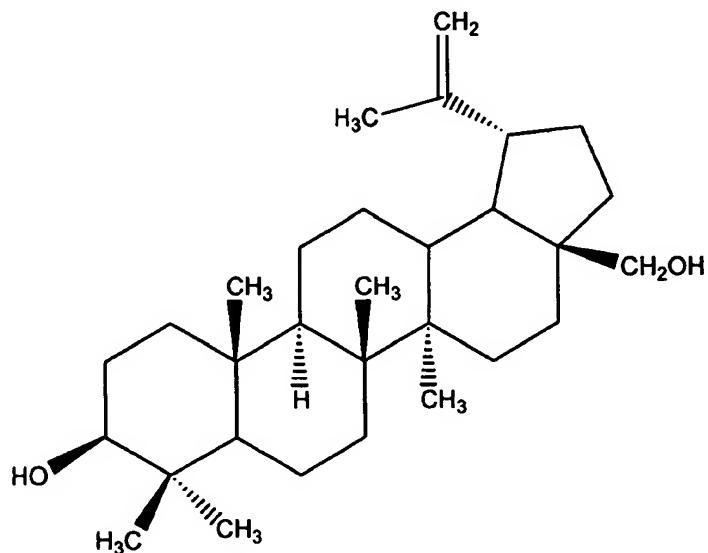
As used herein, “triterpene” or “triterpenoid” refers to a plant secondary metabolite that includes a hydrocarbon, or its oxygenated analog, that is derived from squalene by a sequence of straightforward cyclizations, functionalizations, and sometimes rearrangement. Triterpenes or analogues thereof can be prepared by methods known in the art, i.e., using conventional synthetic techniques or by isolation from plants.

25 Suitable exemplary triterpenes and the biological synthesis of the same are disclosed, e.g., in R.B. Herbert, The Biosynthesis of Secondary Plant Metabolites, 2nd. ed. (London: Chapman 1989). The term “triterpene” refers to one of a class of compounds having approximately 30 carbon atoms and synthesized from six isoprene units in plants

30 and other organisms. Triterpenes consist of carbon, hydrogen, and optionally oxygen. Most triterpenes are secondary metabolites in plants. Most, but not all, triterpenes are

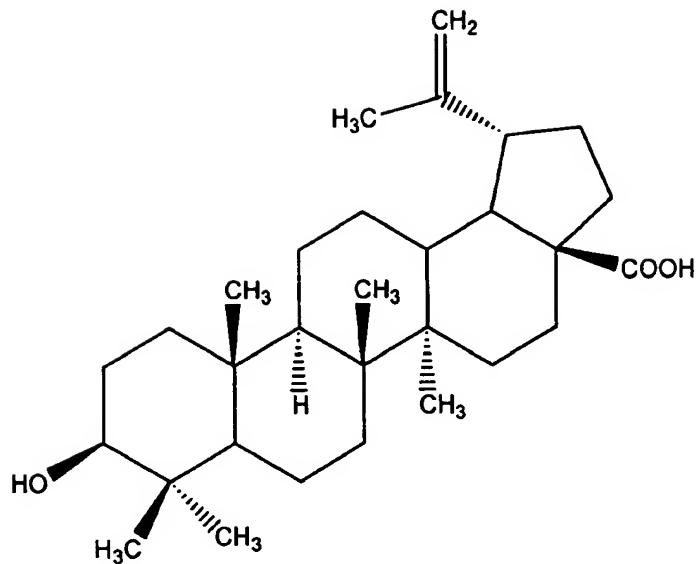
pentacyclic. Examples of triterpenes include betulin, allobetulin, lupeol, friedelin, and all sterols, including lanosterol, stigmasterol, cholesterol,  $\beta$ -sitosterol, and ergosterol.

As used herein, “betulin” refers to  $3\beta,28$ -dihydroxy-lup-20(29)-ene. Betulin is a pentacyclic triterpenoid derived from the outer bark of paper birch trees (*Betula papyrifera*, *B. pendula*, *B. verucosa*, etc.). It can be present at concentrations of up to about 24% of the bark of white birch. Merck Index, twelfth edition, page 1236 (1996).  
5 Structurally, betulin is shown below:

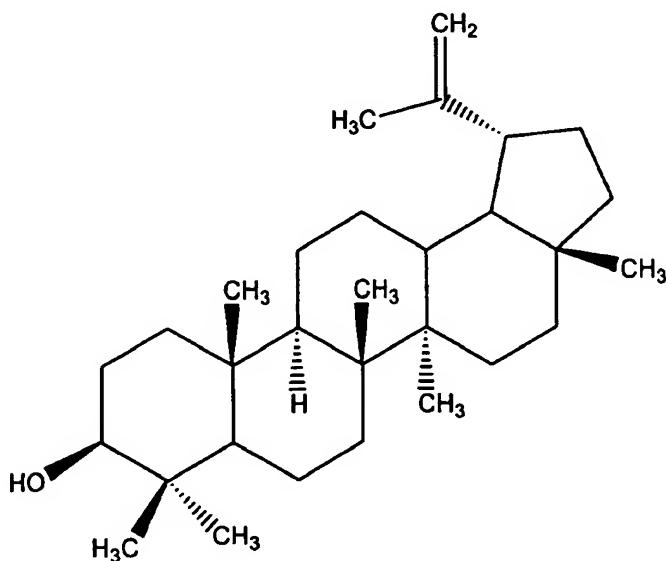


10

As used herein, “betulinic acid” refers to 9-hydroxy-1-isopropenyl-5a,5b,8,8,11a-pentamethyl-eicosahydro-cyclopenta[a]chrysene-3a-carboxylic acid. Structurally, betulinic acid is shown below:



5 As used herein, "lupeol" refers to lup-20 (29)-en-3  $\beta$ -ol. Lupeol is also found in birch bark and in other plant sources. Lupeol is present at concentrations of about 1.5-3% of the birch bark and at up to about 8.2% in *Canavalia ensiformis*, a plant widespread in the humid tropics of Asia and Africa. Structurally, lupeol is shown below:



As used herein, “natural product” refers to naturally occurring compounds that are end products of secondary metabolism; often, they are unique compounds for particular plants or species of plants. In a specific embodiment of the present invention, the compounds can be derived from plant material, e.g., birch bark. Such compounds can 5 include, e.g., triterpenes or triterpenoids. Additionally, the natural product can include a single compound, or can include one or more different compounds. Additionally, these one or more different compounds can be structurally related or structurally unrelated.

As used herein, “plant material” or “plant tissue” refers to a collection of similar cells of a plant, that typically act together to perform a particular function. The term 10 refers to the tissue of any organism of the plant kingdom, as opposed to one of the animal kingdom or of the kingdoms of Fungi, Protista, or Monera. The plant tissue can be any portion or portions of the plant (e.g., bark, roots, leaves, flowers, needles, bulbs, berries, rhizomes, rootstocks, stems, and seeds), as well as the entire plant. The tissues of a plant (“plant tissue”) generally fall into three main categories: dermal tissue, ground tissue, and 15 vascular tissue. Dermal tissue refers to the “skin” layer of all plant organs and is responsible for environmental interaction (light passage, gas exchange, pathogen recognition and protection, color display, etc.). Dermal tissue is composed of epidermal cells, closely packed cells that secrete a waxy cuticle that aids in the prevention of water loss. Ground tissue lies between dermal tissue and vascular tissue. The ground tissue 20 comprises the bulk of the primary plant body. Parenchyma, collenchyma, and sclerenchyma cells are common in the ground tissue. In roots, the ground tissue may store sugars or starches to fuel the spring sap flow; in leaves, the ground tissue is the layer responsible for photosynthesis (the mesophyll). Vascular tissue transports food, water, hormones and minerals within the plant. Vascular tissue includes xylem, phloem, 25 parenchyma, and cambium cells.

As used herein, “bark” refers to the dry, dead outer covering of woody branches, stems and roots of plants that is very distinct and separable from the wood itself. It includes all tissue outside the cambium (growth layer between bark and wood).

As used here the terms “leaf” or “leaves” refer to those parts of a plant which grow along 30 the sides of branches or stems or at the bases of plants. Most are green and contain chlorophyll, though they vary in their shapes and sizes. Leaves are the part of the plant

that ordinarily performs photosynthesis (the process that converts sunlight and carbon dioxide into energy).

As used herein, "needle" generally refers to a narrow stiff leaf, such as those of conifers (e.g., pine trees).

5 As used herein, "root" refers to the part of a plant, normally underground, that absorbs nutrients and anchors the plant into the ground.

As used herein, "bulb" refers to a spheroidal body growing from a plant either above or below the ground (usually below), which is usually a bud, consisting of a cluster of partially developed leaves, and producing, as it grows, a stem above, and roots below, 10 (e.g., the onion or tulip bulb). A true bulb is a complete package containing next year's plant (flower) already forming inside. The contents of the bulb are often enclosed in protective, fleshy scales, which are held together by a small basal plate. The scales are modified leaves that contain enough nutrients to sustain the plant through dormancy and early growth. They may be loose and open like those of a lily, or tightly closed like those 15 of a hyacinth. In many bulbs, a paper-thin tunic protects the scales (lilies don't have a tunic). Roots will grow from the bulb's basal plate.

As used herein, "berry" refers to any small fruit that is pulpy or succulent throughout, having seeds loosely imbedded in the pulp, such as the currant, grape, or blueberry. Berry can be further defined as an indehiscent fruit derived from a single 20 ovary and having the whole wall fleshy, such as the grape or tomato. Furthermore, berries come in various structures including simple, such grape; blueberry, cranberry, or aggregate, such as blackberry; raspberry, strawberry mulberry.

As used herein, "rhizome" refers to a horizontal, usually underground stem that often sends out roots and shoots from its nodes (also called rootstalk or rootstock).

25 As used herein, "rootstock" refers to a robust plant that provides the root system in grafting, also known as a stock. Scions and buds are grafted and budded to a rootstock or stock. Rootstock also refers to the elongated and often thick rhizomes of certain perennial herbaceous plants such as the Iris, Aspidistra and Solomon's Seal.

As used herein, "stem" refers to the main (usually aerial) axis (sometimes referred 30 to as the trunk or stalk) of a tree, shrub, or plant. "Stem" also refers to the part of the

plant that supports the leaves, flowers or fruits of a plant, such as the peduncle of a fruit or the pedicel of a flower.

As used herein, "seed" refers to a ripened ovule, consisting of an embryo with one or more integuments, or coverings, such as an apple seed, a currant seed, dill seed, or 5 kola nut seed. By germination, most seeds produce a new plant. "Seed" also refers to any small seedlike fruit, though it may consist of a pericarp, or even a calyx, as well as the seed proper, such as a parsnip seed or thistle seed. The seed proper has an outer and an inner coat, and within these the kernel or nucleus. The kernel is either the embryo alone, or the embryo enclosed in the albumen, which is the material for the nourishment 10 of the developing embryo. The scar on a seed, left where the stem parted from it, is called the hilum, and the closed orifice of the ovule, the micropyle.

As used herein, "separating" refers to the process of removing solids from a mixture. The process can employ any technique known to those of skill in the art, e.g., decanting the mixture, filtering the solids from the mixture, or a combination thereof.

15 As used herein, "filtering" refers to the process of removing solids from a mixture by passing the liquid through a filter, thereby suspending the solids on the filter.

As used herein, "decanting" refers to the process of pouring off a liquid without disturbing the sediment, or the process of pouring off a liquid with a minimal disturbance of the sediment.

20 As used herein, "aqueous base" refers to a solution of water, and a substance that produces  $\text{OH}^-$  ions in the aqueous solution. Specifically, the aqueous base can include water and at least one of a lithium ion ( $\text{Li}^+$ ), a sodium ion ( $\text{Na}^+$ ), a potassium ion ( $\text{K}^+$ ), a calcium ion ( $\text{Ca}^{2+}$ ), and a barium ion ( $\text{Ba}^+$ ). More specifically, the aqueous base can include water and at least one of sodium hydroxide ( $\text{NaOH}$ ) and potassium hydroxide 25 ( $\text{KOH}$ ).

As used herein, "alkaline metal" refers to metals of Group IA of the Periodic Table of Elements, e.g., sodium ( $\text{Na}$ ) and potassium ( $\text{K}$ ).

As used herein, "alkaline earth metal" refers to metals of Group IIA of the Periodic Table of Elements, e.g., magnesium ( $\text{Mg}$ ) and calcium ( $\text{Ca}$ ).

30 As used herein, "water-immiscible solvent" refers to a solvent that is not miscible (i.e., not capable of mixing in all proportions) with water. Suitable specific water-

immiscible solvents include, e.g., aromatic hydrocarbons such as xylenes, *o*-xylene, *m*-xylene, *p*-xylene, toluene, benzene, and combinations thereof; chlorinated solvents such as chloroform and methylene chloride; as well as other organic solvents such as acetone and ethyl acetate.

5 As used herein, "water-miscible solvent" refers to a solvent that is miscible (i.e., is capable of mixing in all proportions) with water. Suitable specific water-miscible solvents include, e.g., methanol, ethanol, *iso*-propanol, *tert*-butanol, ethylene glycol, and propylene glycol.

10 As used herein, "distill" or "distillation" refers to the process of extracting the volatile components of a mixture by the condensation and collection of the vapors that are produced as the mixture is heated. The process includes the evaporation and subsequent collection of a liquid by condensation.

15 As used herein, "concentrating" or "condensing" refers to the process whereby the volume is reduced, by the removal of liquid.

As used herein, "mother liquor" refers to the liquid obtained after solids are removed from a mixture or a solution of solids in a liquid. As such, the mother liquor will not include an appreciable amount of these solids.

20 As used herein, "birch" refers to any of the several deciduous trees of the genus *Betula*. The birches comprise the family *Betulaceae* in the order *Fagales*. Birch trees include, for example, white birch, *B. alba*; sweet, black or cherry birch, *B. lenta*; monarch birch, *B. Maximowicziana*; dwarf or arctic birch, *B. Nana*; Japanese white birch, *B. Platyphylla Japonica*; smooth-bark birch, *B. Pubescens*; yellow birch, *B. alleghaniensis*; paper, white or canoe birch, *B. papyrifera*; grey birch, *B. populifolia*; river birch, *B. nigra*; and the European birches, *B. pubescens*; *B. Alba* and *B. pendula*. Specifically, the 25 birch can be *B. alba*, *B. lenta*, *B. Maximowicziana*, *B. Nana*, *B. Platyphylla Japonica*, *B. Pubescens*, *B. alleghaniensis*, *B. papyrifera*, *B. populifolia*, *B. nigra*, *B. pubescens*, *B. Alba* or *B. pendula*. A specific birch for use in the processes of the present invention is *B. papyrifera*.

30 As used herein, "birch bark" refers to inner birch bark and outer birch bark. Inner birch bark is more dense and granular than outer birch bark, while outer birch bark is more flexible and fibrous than inner birch bark. Outer birch bark is light in color, thin (1-

5 mm), tough, and of low water-content relative to inner birch bark. The inner bark is darker in color, thicker (3-10 mm) and non-fibrous relative to the outer bark. The inner bark is the portion of the tree wherein significant water transport occurs (i.e., an area of high water content). Due to the differences in the physical properties of inner birch bark 5 and outer birch bark, fragmentation produces outer birch bark shreds and inner birch bark chunks.

Outer birch bark shreds can be separated from the inner birch bark chunks using any suitable means. The separation can conveniently be accomplished by screening the mixture through a mesh having openings intermediate in size between the smaller inner 10 inner bark chunks and the larger outer bark shreds. The smaller inner bark chunks fall through the screen and are separated from the outer bark.

The "mesh" can be a unit comprising one or more open spaces in a cord, thread, or wire network in which the cords, threads or wires surround the spaces. Any mesh suitable to separate inner birch bark from outer birch bark can be employed. Typically, 15 the mesh is a wire mesh containing openings of about  $\frac{1}{2}$  of an inch by  $\frac{1}{2}$  of an inch, or smaller. For example, mesh can conveniently contain openings of about  $\frac{1}{4}$  of an inch by about  $\frac{1}{4}$  of an inch. Specifically, the size of the mesh can be about 20 mm by about 20 mm, or about 10 mm by about 10 mm, or about 6 mm by about 6 mm. More specifically, the size of the mesh can be about 3 mm by about 3 mm.

20 Alternatively, the inner birch bark chunks and outer birch bark shreds may be separated with the use of an air classifier. As used herein, an "air classifier" is a device which operates on the principle of the differing properties of the two components (e.g., inner and outer birch bark) in an air stream to effect a physical separation. Typically, the less dense outer bark travels a greater distance in the air stream than the more dense inner 25 bark. The inner bark, along with other materials, falls rapidly from the stream of air. As a result, the inner birch bark and the outer birch bark can be separated.

After separating outer birch bark from inner birch bark, outer birch bark of about 10 wt.% to about 45 wt.% based on initial birch bark content is typically obtained and inner birch bark of about 55 wt.% to about 85 wt.% is typically obtained.

30 For use in the processes of the present invention, birch bark shreds less than about 10 mm in diameter can conveniently be used. More specifically, outer birch bark shreds

less than about 6 mm in diameter, less than about 4 mm in diameter, or less than about 2 mm in diameter, can be used. Alternatively, birch bark pellets of about 5.0 mm in length by about 4 mm in diameter, or about 2.5 mm in length by about 2 mm in diameter can conveniently be used. In addition, bark pellets of about 0.25 kg/liter to about 1.0 kg/liter, 5 or about 0.5 kg/liter to about 0.7 kg/liter can conveniently be used.

As used herein, "aromatic hydrocarbon" refers to a compound having at least one phenyl or naphthyl ring, wherein the compound contains carbon and hydrogen atoms. The aromatic hydrocarbon can optionally be substituted, e.g., with one or more groups selected from the group of alkyl (e.g., methyl), hydroxyl, halo, alkoxy, cyano, carboxyl, 10 sulfonyl, and amino. Suitable specific aromatic hydrocarbons include, e.g., xylenes, *o*-xylene, *m*-xylene, *p*-xylene, toluene, benzene, and combinations thereof.

As used herein, "reflux" refers to the process of boiling a liquid in a vessel attached to a condenser so that the vapors continuously condense for reboiling.

As used herein, "washing" refers to the process of purifying a solid mass (e.g., 15 crystals) by passing a liquid over and/or through the solid mass, as to remove soluble matter. The process includes passing a solvent, such as distilled water, over and/or through a precipitate obtained from filtering, decanting, or a combination thereof.

As used herein, a "binder" refers to a substance that can effectively bind (i.e., chemically and/or physically attach itself) to selective compound(s) present in a mixture. 20 It is believed that substances such as aluminum alcoholates bind strongly and irreversibly to triterpenoid or triterpene acids and tannins, therefore providing complete discoloration of crude birch bark extract. Suitable binders useful in the present invention include, e.g., metal hydrides (e.g., lithium hydride (LiH), sodium hydride (NaH), potassium hydride (KH), calcium hydride (CaH<sub>2</sub>), lithium aluminum hydride (LiAlH<sub>4</sub>), and boron aluminum 25 hydride (BAIH<sub>4</sub>)); metal alcoholates (e.g., sodium methoxide (NaOMe), sodium ethoxide (NaOEt), potassium methoxide (KOMe), potassium ethoxide (KOEt), aluminum *iso*-propoxide [Al(i-OPr)<sub>3</sub>], aluminum *tert*-butoxide [Al(t-OBu)<sub>3</sub>], and aluminum methoxide [Al(OMe)<sub>3</sub>]), ortho-esters (e.g., ethylorthocarbonate) and dialkoxy sulfates (e.g., dimethylsulfate and diethylsulfate).

30 As used herein, "metal hydride" refers to a binary compound of hydrogen and a metal. Suitable metal hydrides include e.g., lithium hydride (LiH), sodium hydride

(NaH), potassium hydride (KH), calcium hydride (CaH<sub>2</sub>), lithium aluminum hydride (LiAlH<sub>4</sub>), and boron aluminum hydride (BAIH<sub>4</sub>).

As used herein, “metal alcoholate” or “alcoholate” refers to an organic alcohol wherein the hydroxy hydrogen has been replaced with a metal, e.g., (CH<sub>3</sub>CH<sub>2</sub>O)<sub>3</sub>Al.

5      Aluminum alcoholates are suitable reagents for triterpene purification because it is believed that aluminum alcoholates bind strongly and irreversibly to acids and tannins, therefore providing complete discoloration of the total extract. Suitable specific metal alcoholates include, e.g., sodium methoxide (NaOMe), sodium ethoxide (NaOEt), potassium methoxide (KOMe), potassium ethoxide (KOEt), aluminum *iso*-propoxide [Al(i-OPr)<sub>3</sub>], aluminum *tert*-butoxide [Al(t-OBu)<sub>3</sub>], and aluminum methoxide [Al(OMe)<sub>3</sub>].

10     [Al(OMe)<sub>3</sub>].

15     As used herein, “hydrolyze” or “hydrolysis” refers to the process of converting a carboxylic ester to the corresponding carboxylic acid, with the addition of water. The reaction (i.e., hydrolysis) can conveniently be carried out employing any suitable reagent(s) and reaction condition(s). For example, the reaction can be carried out in a suitable solvent and at a suitable temperature and pressure, under basic conditions, neutral conditions, or acidic conditions. Suitable reagents and reaction conditions are disclosed, e.g., in Advanced Organic Chemistry, Part B: Reactions and Synthesis, Carey and Sundberg, Plenum Press; Comprehensive Organic Transformations, Larock, Wiley & Sons; and Advanced Organic Chemistry, Reactions Mechanisms, and Structure, March, McGraw Hill.

20     For example, the hydrolysis can be carried out at a pH of greater than about 7.0 (e.g., a pH of about 7-8, 8-9, 9-10, 10-11, or 11-12). When the hydrolysis is carried out at a pH of greater than 7.0, one or more suitable bases will typically be employed.

25     Suitable bases include metal hydroxides and metal alkoxides. Suitable metal hydroxides include sodium hydroxide, potassium hydroxide, lithium hydroxide, calcium hydroxide and barium hydroxide. Suitable metal alkoxides include lithium methoxide, lithium ethoxide, lithium isopropoxide, lithium *tert*-butoxide, sodium methoxide, sodium ethoxide, sodium isopropoxide, sodium *tert*-butoxide, potassium methoxide, potassium ethoxide, potassium isopropoxide, potassium *tert*-butoxide, magnesium methoxide, magnesium ethoxide, barium methoxide, barium ethoxide, calcium methoxide and

30     calcium ethoxide.

calcium ethoxide. A specific base suitable for the processes of the present invention (i.e., hydrolysis of birch bark) is sodium hydroxide.

Alternatively, the hydrolysis can be carried out at a pH of less than about 7.0 (e.g., a pH of about 1-2, 2-3, 3-4, 4-5, 5-6, or 6-7). When the hydrolysis is carried out at a pH of less than about 7.0, one or more suitable acids will typically be employed. Suitable acids include, for example, hydrochloric acid, phosphoric acid, formic acid, hydrobromic acid, sulfuric acid, nitric acid, acetic acid, and combinations thereof. A specific acid suitable for the processes of the present invention (i.e., hydrolysis of birch bark) is sulfuric acid.

10 Alternatively, the hydrolysis can be carried out at a pH of about 6.5 to about 7.5 (i.e., neutral conditions). When the hydrolysis is carried out at about 6.5 to about 7.5 (i.e., neutral conditions), water at standard pressure or at an elevated pressure can be employed.

15 As used herein, "natural ester" refers to an organic compound (e.g., triterpenes or triterpenoids) having at least one carboxylic ester group.

As used herein, "azeotropic distillation" refers to the process of boiling off or distilling any liquid mixture having constant minimum and maximum boiling points and distilling off without decomposition and in a fixed ratio, as with benzene and water, or as with toluene and water. In a specific embodiment, the azeotropic distillation can include 20 the distillation of water and at least one of xylenes, *o*-xylene, *m*-xylene, *p*-xylene, toluene, and benzene. Such a distillation can be carried out at a temperature and a period of time, effective to distill or boil more than about 90% of the water, more than about 95% of the water, more than about 98% of the water, or up to about 100 wt.% of the water. With the distillation or boiling off of the water, it is appreciated that those of skill 25 in the art recognize that a discrete amount of the organic solvent (e.g., xylenes, *o*-xylene, *m*-xylene, *p*-xylene, toluene, and/or benzene) will also distill or boil off with the water.

As used herein, "agitating" refers to the process of putting a mixture into motion with a turbulent force. Suitable methods of agitating include, e.g., stirring, mixing, and shaking.

As used herein, "precipitating" refers to the process of causing a solid substance (e.g., crystals) to be separated from a solution. The precipitating can include, e.g., crystallizing.

As used herein, "polar organic solvent" refers to an organic solvent having a  
5 measurable dipole.

As used herein, "non-polar organic solvent" refers to an organic solvent having no measurable dipole.

As used herein, "drying" refers to the removal of water and/or solvent, such that the water and/or solvent content is below about 5 wt.%, below about 2 wt.% or below  
10 about 1 wt.%.

As used herein, "neutralizing" refers to the process of changing or bringing the pH to about  $7 \pm 1$ . As such, the neutralizing can include bringing the pH to about 6 to about 8. In a specific embodiment, the neutralizing can include bringing the pH to about 6.5 to about 7.5 (i.e., to a pH of about  $7 \pm 0.5$ ). In yet another specific embodiment, the  
15 neutralizing can include bringing the pH to about 6.75 to about 7.25 (i.e., to a pH of about  $7 \pm 0.25$ ).

As used herein, "acidifying" refers to the process of lowering the pH to below 7.0. For example, in one specific embodiment, the acidifying includes lowering the pH to below about 6.0. In another specific embodiment, the acidifying includes lowering the  
20 pH to below about 5.0. In yet another specific embodiment, the acidifying includes lowering the pH to below about 4.0.

As used herein, "purifying" refers to the process of ridding a solid substrate (e.g., crystals) of impurities. Suitable methods of purifying include, e.g., washing, recrystallizing and drying.

25 References in the specification to "one embodiment", "an embodiment", "an example embodiment", etc., indicate that the embodiment described may include a particular feature, structure, or characteristic, but every embodiment may not necessarily include the particular feature, structure, or characteristic. Moreover, such phrases are not necessarily referring to the same embodiment. Further, when a particular feature,  
30 structure, or characteristic is described in connection with an embodiment, it is submitted that it is within the knowledge of one skilled in the art to affect such feature, structure, or

characteristic in connection with other embodiments whether or not explicitly described.

Referring to **Figure 1**, birch bark processing (1) utilizing azeotropic distillation (6) is illustrated. The processing (1) provides for the isolation of betulinic acid (15), betulin (17), and/or lupeol (20). The processing includes extracting (3) outer birch bark (2) with an organic solvent to provide a birch bark extract (4). Suitable solvents, techniques and conditions for the extracting (3) are known to those of skill in the art. The extracting (3) will preferably employ suitable solvent(s) in which the desired compound(s) are relatively soluble. The extracting (3) can be carried out under conditions (e.g., temperature, pressure and period of time), effective to extract the desired compound(s) in a relatively high yield, while minimizing any decomposition of the desired compound(s).

The extracting (3) effectively provides birch bark extract (4), which can subsequently be refluxed (21) with a metal hydroxide (5), azeotropically distilled (6), and filtered/decanted (7), to provide the solids (8) and filtrate (9). The refluxing (21) of the birch bark extract (4) with the metal hydroxide (5) can be carried out under conditions (e.g., temperature and period of time) and with reagents and solvents, effective to reflux (21) the reaction mixture, while minimizing any decomposition of the desired compound(s). Suitable metal hydroxides (5) are known to those of skill in the art.

The reaction mixture can then be azeotropically distilled (6), to effectively remove water present in the reaction mixture. This distillation (6) will remove not only water, but also a discrete amount of organic solvent (e.g., xylenes, toluene and/or benzene) present in the reaction mixture. The distillation (6) will also not remove any appreciable amounts of metal hydroxides (5) along with the water. Additionally, no appreciable amounts of triterpenes or triterpenoids are removed with the water during the distillation (6). It is believed that the distillation (6), upon removal of the water, causes the metal hydroxide (5) to precipitate from solution, due to its relative insolubility in the organic solvent. As the metal hydroxide (5) precipitates from solution, the metal hydroxide (5) reacts with the triterpenoid acids present in solution, forming a solid (8), which can be visible as a black tar. This solid (8) precipitates from solution, and can subsequently be separated (e.g., filtered or decanted (7)) from solution to provide the solid (8) and filtrate (9). As discussed below, the solid (8) contains betulinic acid (15),

and can be purified accordingly.

The filtrate (9) can be concentrated (13) and crystallized (14), to provide betulin (17) and a filtrate (16). This filtrate (16) can be concentrated (18) and crystallized (19) to provide lupeol (20).

5 The solid (8) described above can be neutralized (10) or acidified, filtered or decanted (11), and subsequently crystallized (12) to provide betulinic acid (15).

The present invention will be described by the following examples. The examples are for illustration purposes and do not otherwise limit the invention.

10

### Examples

#### Example 1: Extraction of outer birch bark with xylenes

15 Air-dry pellets of outer birch bark (200 g) were extracted with p-xylene (1 x 1 L) and then with p-xylene (3 x 300ml). Each time the extraction mixture was refluxed for 20 min and then filtered at 90°C. The solvent of the combined organic extracts was evaporated and the residue was dried at 80°C, *in vacuum*, to give 44 g (yield is 22 wt.%) of dry birch bark extract. The p-xylene extract was used without further purification.

20

#### Example 2: Isolation of betulin

25 The birch bark extract (44 g) obtained from the procedure in Example 1 was dissolved in xylenes (1.1 L) at 80°C. Sodium hydroxide (NaOH) (8.8g) was dissolved in 80°C water (17.4 ml). The two mixtures were combined, vigorously stirred and refluxed for 1 hr. Water (17.4 ml) was removed from the reaction mixture by azeotropic distillation on a Dean-Stark trap, and the reaction mixture was filtered to remove the black tar that precipitated from solution. This black tar includes the crude sodium salts of triterpenoid acids (19.8 g), including betulinic acid, as shown in Example 4 below. To the mother liquor xylene solution obtained after filtration was added aluminum *iso*-  
30 propoxide [Al(Oi-Pr)<sub>3</sub>] (2.64 g, 12.9 mmol) and stirred for 1hr at 85°C. Alumina oxide (Al<sub>2</sub>O<sub>3</sub>) (4.4 g, acidic Brockman I) was added and the reaction mixture was stirred for 4

hrs at 85°C, filtered and 800 ml of xylenes was evaporated. The resulting solution was cooled to room temperature and left undisturbed for 10 hrs. The crystals were filtered, dried and 21 g (47.7 wt.%, based on the birch bark extract) of betulin (98% purity) was obtained. The concentration of betulinic acid in this betulin was less than 0.02 wt.%.

5

**Example 3: Isolation of lupeol**

The mother liquid from Example 2 was evaporated to afford 11 g (25 %) of crude lupeol / betulin mixture. 20 ml of acetone was added to this mixture, stirred at 60°C for on hour and cooled down 5-10°C for two hours. The yellowish precipitate (4.5 g) was 10 filtered and crystallized from xylene. The mother liquid after xylene filtering was removed and the yellowish solid crystals of lupeol were crystallized from cyclohexane. The mother liquid after cyclohexane filtering was removed and the solids were washed with 5 ml of acetone. 1.1g of white lupeol crystals (98%+ purity) were obtained (yield is 2.5 wt.%, based on birch bark extract).

15

**Example 4: Isolation of betulinic acid**

19.8 g the sodium salts of triterpenoid acids obtained in Example 2 above was washed with water (2 x 50ml). The water solution was acidified with a 5% solution of HCl in water (to pH=5), filtered and dried to give 2.5 g of crude product. The crude 20 product was then dissolved in 80 ml of *iso*-propanol (i-PrOH), and 65 ml of *iso*-propanol (i-PrOH) was evaporated and filtered at 50°C. The crystals were dried to provide 1.3 g (3 wt.%, based on birch bark extract) of betulinic acid (90 % purity). After a second crystallization from *iso*-propanol (i-PrOH) 0.9 g (2.0 wt.%, based on birch bark extract) of betulinic acid (99% purity) was obtained.

25

All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made 30 while remaining within the spirit and scope of the invention.

Claims

1. A method for obtaining a natural product from plant material, the method  
5 comprising:
  - (a) contacting plant material with a solvent to provide a first mixture;
  - (b) separating the plant material from the solvent to provide a first extract;
  - (c) contacting the first extract with an aqueous base to provide a second mixture;
  - (d) heating the second mixture in a water-immiscible solvent, effective to distill  
10 off water present in the second mixture, thereby providing a third mixture;
  - (e) separating solids from the third mixture to provide a fourth mixture; and
  - (f) concentrating the fourth mixture, or precipitating solids from the fourth mixture, to provide a natural product.
- 15 2. The method of claim 1 wherein more than one natural product is obtained.
3. The method of claim 1 wherein at least one of betulin, betulinic acid, and lupeol  
are obtained.
- 20 4. The method of claim 1 wherein the natural product obtained is betulin, in a purity  
of at least about 95 wt.%.
5. The method of claim 1 wherein the natural product obtained is betulin, and  
wherein the betulin includes betulinic acid in a weight ratio of at least about 5,000:1, of  
25 betulin to betulinic acid.
6. The method of claim 1 wherein the natural product obtained is betulin, and  
wherein the betulin includes betulinic acid in a weight ratio of at least about 10,000:1, of  
betulin to betulinic acid.

7. The method of claim 1 wherein the natural product obtained is betulin, and wherein the betulin includes betulinic acid in an amount of up to about 0.02 mol. %.
8. The method of claim 1 wherein the precipitating of the solids from the fourth mixture provides the natural product and a mother liquor.
9. The method of claim 1 wherein the precipitating of the solids from the fourth mixture provides betulin, and a mother liquor that comprises lupeol.
10. 10. The method of claim 1 wherein, in step (e), the solids separated from the third mixture comprise betulinic acid.
11. The method of claim 1 wherein the plant material employed comprises birch bark.
15. 12. The method of claim 1 wherein the plant material employed comprises inner birch bark.
13. The method of claim 1 wherein the plant material employed comprises outer birch bark.
20. 14. The method of claim 1 wherein the plant material employed comprises birch bark pellets.
25. 15. The method of claim 1 wherein the plant material employed comprises at least about 150 kg of birch bark.
16. The method of claim 1 wherein the plant material employed comprises at least about 2,000 kg of birch bark.
30. 17. The method of claim 1 wherein the plant material employed comprises *Betula papyrifera*.

18. The method of claim 1 wherein the plant material employed comprises *Betula pendula*.

5 19. The method of claim 1 wherein the water-immiscible solvent in step (d) comprises an optionally substituted aromatic hydrocarbon.

20. The method of claim 1 wherein the water-immiscible solvent in step (d) comprises at least one of xylene, toluene, and benzene.

10 21. The method of claim 1 wherein the contacting in step (a) is further accompanied by heating the first mixture.

22. The method of claim 1 wherein the contacting in step (a) is further accompanied by heating the first mixture above about 90°C.

15 23. The method of claim 1 wherein the contacting in step (a) is further accompanied by heating the first mixture to reflux.

20 24. The method of claim 1 wherein the contacting in step (a) is further accompanied by heating the first mixture for more than about 30 minutes.

25. The method of claim 1 wherein the separating in step (b) includes filtering the first mixture, decanting the first mixture, or a combination thereof.

25 26. The method of claim 1 wherein the separating in step (b) comprises filtering the first mixture, decanting the first mixture, or a combination thereof; wherein the temperature of the first mixture is above about 70°C.

27. The method of claim 1 wherein the separating in step (b) comprises filtering the first mixture, decanting the first mixture, or a combination thereof; and washing any solids obtained with a water-immiscible solvent.

5 28. The method of claim 1 wherein the separating in step (b) comprises filtering the first mixture, decanting the first mixture, or a combination thereof; and washing any solids obtained with a water-immiscible solvent having a temperature of up to about 110°C.

10 29. The method of claim 1 wherein the separating in step (b) comprises filtering the first mixture, decanting the first mixture, or a combination thereof; and washing any solids obtained with xylenes.

15 30. The method of claim 1 further comprising, after step (b), concentrating the first extract.

31. The method of claim 1 wherein the aqueous base comprises an alkaline metal or an alkaline earth metal.

20 32. The method of claim 1 wherein the aqueous base comprises at least one of a lithium ion ( $\text{Li}^+$ ), a sodium ion ( $\text{Na}^+$ ), a potassium ion ( $\text{K}^+$ ), a calcium ion ( $\text{Ca}^{2+}$ ), and a barium ion ( $\text{Ba}^+$ ).

25 33. The method of claim 1 wherein the aqueous base comprises at least one sodium hydroxide ( $\text{NaOH}$ ) and potassium hydroxide ( $\text{KOH}$ ).

34. The method of claim 1 wherein the second mixture further comprises a binder selected from the group of metal hydrides, metal alcoholates, ortho-esters and dialkoxy sulfates, and combinations thereof.

35. The method of claim 1 wherein the second mixture further comprises a binder selected from the group of lithium hydride (LiH), sodium hydride (NaH), potassium hydride (KH), calcium hydride (CaH<sub>2</sub>), lithium aluminum hydride (LiAlH<sub>4</sub>), and boron aluminum hydride (BAIH<sub>4</sub>).

5

36. The method of claim 1 wherein the second mixture further comprises a binder selected from the group of sodium methoxide (NaOMe), sodium ethoxide (NaOEt), potassium methoxide (KOMe), potassium ethoxide (KOEt), aluminum *iso*-propoxide [Al(i-OPr)<sub>3</sub>], aluminum *tert*-butoxide [Al(t-OBu)<sub>3</sub>], and aluminum methoxide [Al(OMe)<sub>3</sub>].

10

37. The method of claim 1 wherein the second mixture further comprises a binder selected from the group of ethylorthocarbonate, dimethylsulfate, and diethylsulfate.

15

38. The method of claim 1 wherein, in step (d), the second mixture is heated at a temperature and for a period of time, effective to distill off water present in the second mixture and to hydrolyze natural esters of the natural product.

20

39. The method of claim 1 wherein, in step (d), the second mixture is heated to reflux.

40. The method of claim 1 wherein, in step (d), the water is distilled off azeotropically with the water-immiscible solvent.

25

41. The method of claim 1 wherein, in step (d), up to about 100 wt.% of the water is azeotropically distilled off.

42. The method of claim 1 wherein, in step (d), more than about 95 wt.% of the water is azeotropically distilled off.

30

43. The method of claim 1 wherein, in step (d), the heating is further accompanied by agitating the second mixture.

44. The method of claim 1 wherein, in step (d), the heating is further accompanied by vigorously stirring the second mixture.

5 45. The method of claim 1 wherein, in step (d), the heating is further accompanied by a the presence of a binder.

46. The method of claim 1 wherein the solvent in step (a) is water-miscible, and the water-miscible solvent is subsequently removed, prior to the heating in step (d).

10 47. The method of claim 1 wherein the solvent in step (a) is water-immiscible, and the water-immiscible solvent is present during the heating, in step (d).

48. The method in claim 1 wherein the separating comprises filtering, decanting, or a 15 combination thereof.

49. The method in claim 1 wherein, in step (e), the solids that are separated from the third mixture comprise betulinic acid.

20 50. The method of claim 1 wherein the separating in step (e) includes filtering the third mixture, decanting the third mixture, or a combination thereof.

51. The method of claim 1 wherein the separating in step (e) includes filtering the third mixture, decanting the third mixture, or a combination thereof; wherein the 25 temperature of the third mixture is above about 70°C.

52. The method of claim 1 wherein the separating in step (e) includes filtering the third mixture, decanting the third mixture, or a combination thereof; and washing any solids obtained with a water-immiscible solvent.

53. The method of claim 1 wherein the separating in step (e) includes filtering the third mixture, decanting the third mixture, or a combination thereof; and washing any solids obtained with a water-immiscible solvent having a temperature of up to about 110°C.

5

54. The method of claim 1 wherein the separating in step (e) includes filtering the third mixture, decanting the third mixture, or a combination thereof; and washing any solids obtained with xylenes.

10 55. The method of claim 1 wherein, in step (f), the concentrating of the fourth mixture is carried out at a pressure of up to about 1 atm.

56. The method of claim 1 wherein the amount of natural product obtained, based on the amount of the first extract obtained in step (b), is at least about 65 wt.%.

15

57. The method of claim 1 that provides about 40 wt.% to about 65 wt.% of betulin, based upon the amount of concentrated first extract.

20 58. The method of claim 1 that provides about 10 wt.% to about 25 wt.% of betulin, based upon the amount of the plant material employed.

59. The method of claim 1 that provides about 2 wt.% to about 5 wt.% of lupeol, based upon the amount of concentrated first extract.

25 60. The method of claim 1 that provides about 2 wt.% to about 5 wt.% of betulinic acid, based upon the amount of concentrated first extract.

61. A method for obtaining betulin from birch bark, the method comprising:

(a) contacting birch bark with a solvent to provide a first mixture;

30 (b) separating the birch bark from the solvent to provide a first extract;

(c) contacting the first extract with an aqueous base to provide a second mixture;

(d) heating the second mixture in a water-immiscible solvent, effective to distill off water present in the second mixture, thereby providing a third mixture;

(e) separating solids from the third mixture to provide a fourth mixture; and

(f) concentrating the fourth mixture to provide betulin, or precipitating betulin

5 from the fourth mixture.

62. A method for obtaining lupeol from birch bark, the method comprising:

(a) contacting birch bark with a solvent to provide a first mixture;

(b) separating the birch bark from the solvent to provide a first extract;

10 (c) contacting the first extract with an aqueous base to provide a second mixture;

(d) heating the second mixture in a water-immiscible solvent, effective to distill off water present in the second mixture, thereby providing a third mixture;

(e) separating solids from the third mixture to provide a fourth mixture;

(f) precipitating solids from the fourth mixture;

15 (g) separating the solids from the fourth mixture to provide a mother liquor; and

(h) concentrating the mother liquor to provide lupeol;

(i) optionally washing the lupeol with a polar organic solvent; and

(j) optionally drying the lupeol.

20 63. A method for obtaining betulinic acid from birch bark, the method comprising:

(a) contacting birch bark with a solvent to provide a first mixture;

(b) separating the birch bark from the solvent to provide a first extract;

(c) contacting the first extract with an aqueous base to provide a second mixture;

(d) heating the second mixture in a water-immiscible solvent, effective to distill

25 off water present in the second mixture, thereby providing a third mixture;

(e) separating solids from the third mixture;

(f) neutralizing or acidifying the solids in an aqueous acid, thereby providing a fourth mixture;

(g) separating betulinic acid from the fourth mixture;

30 (h) optionally washing the betulinic acid with a polar organic solvent; and

(i) optionally drying the betulinic acid.

64. A method for purifying an extract of a natural product, the method comprising:

- (i) contacting an extract of a natural product with an aqueous base, to provide a first mixture;
- 5 (ii) heating the first mixture in a water-immiscible solvent, effective to distill off water present in the first mixture, thereby providing a second mixture;
- (iii) separating solids from the second mixture, to provide a third mixture; and
- (iv) concentrating the third mixture or precipitating solids from the third mixture, to provide a purified natural product.

10

65. The compound obtained from the method of any one of claims 1-64.

66. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the compound of claim 65.

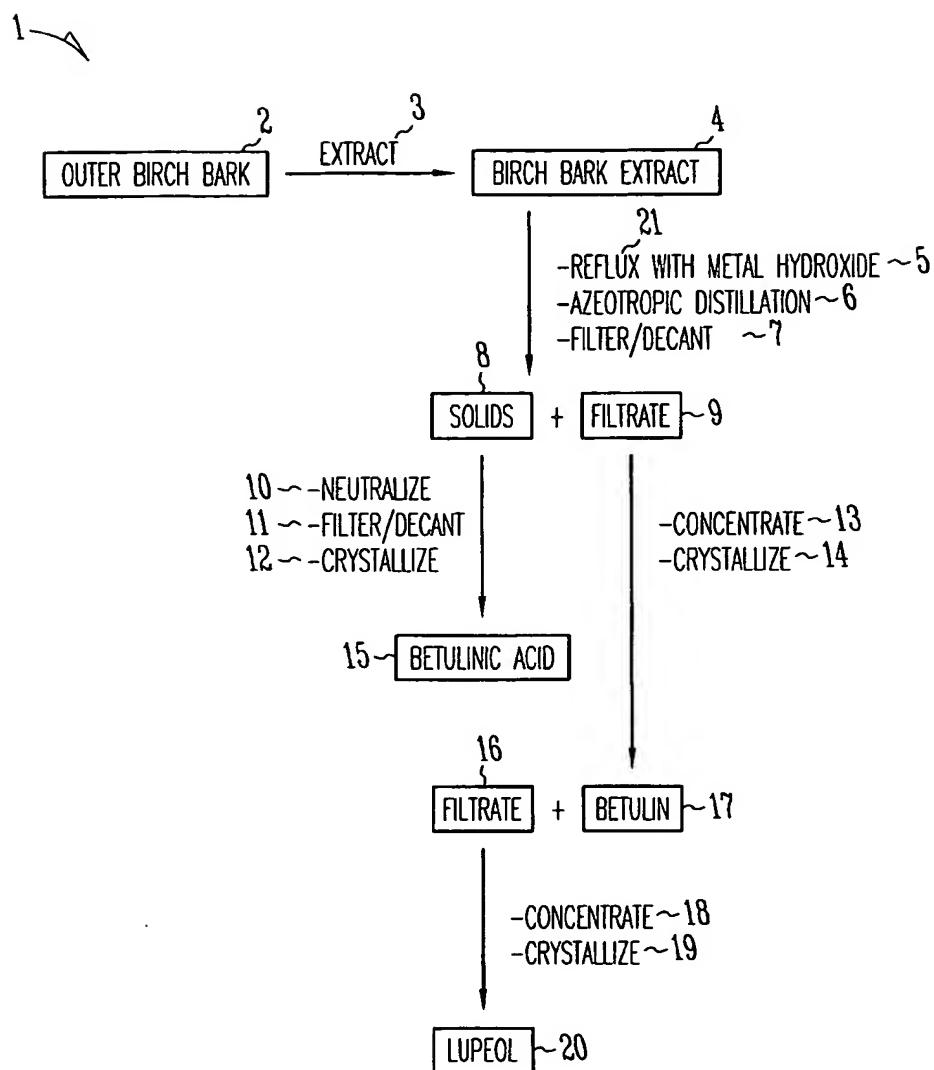
15

67. A cosmetic composition comprising a cosmetically acceptable carrier and the compound of claim 65.

Abstract

The present invention provides a method for obtaining a natural product (e.g., betulin, lupeol and/or betulinic acid) from plant material (e.g., birch bark). The method employs an azeotropic distillation with water, a water-immiscible solvent (e.g., toluene) and an aqueous base (e.g., KOH).

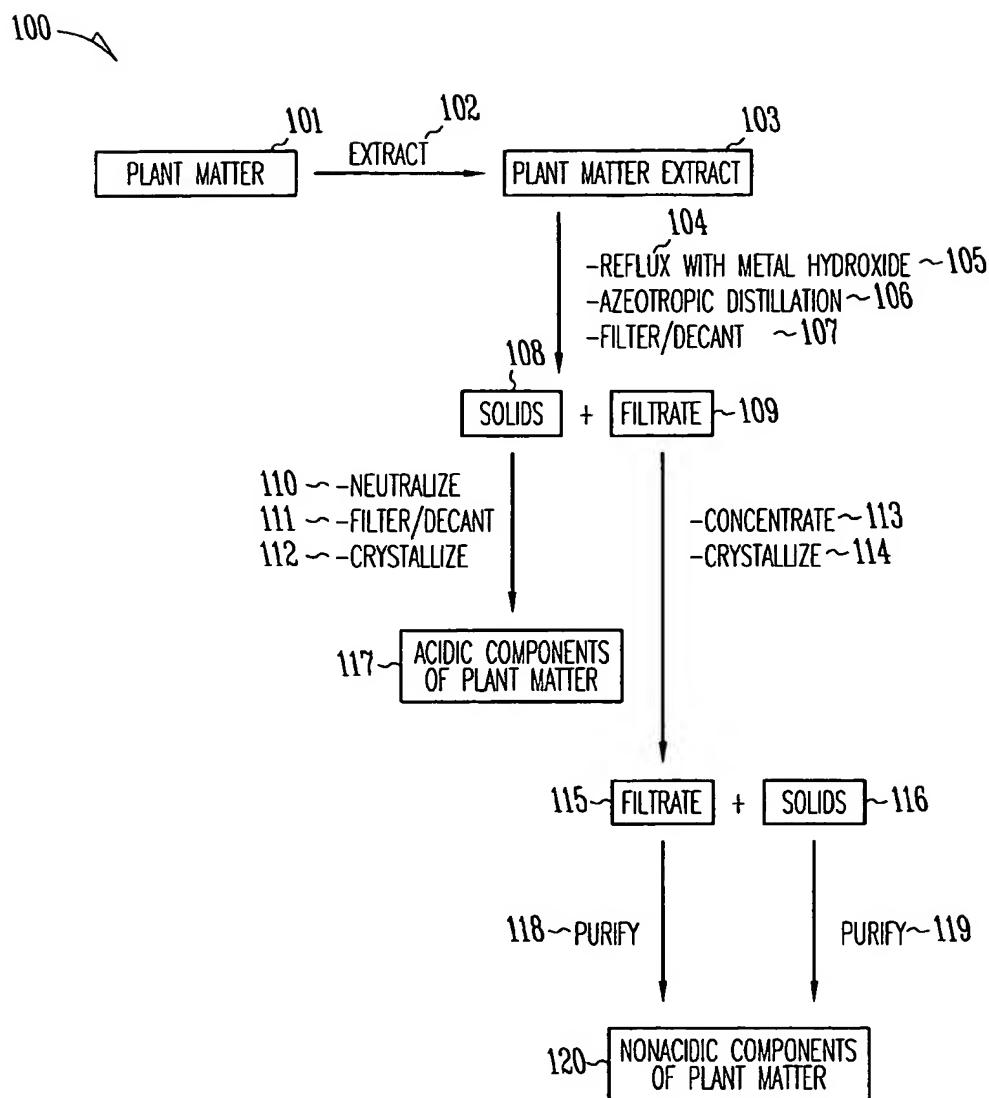
1/2



BIRCH BARK PROCESSING UTILIZING AZEOTROPIC DISTILLATION

*Fig. 1*

2/2



PLANT MATTER PROCESSING UTILIZING AZEOTROPIC DISTILLATION

Fig.2

# Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/038252

International filing date: 10 November 2004 (10.11.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US  
Number: 60/518,988  
Filing date: 10 November 2003 (10.11.2003)

Date of receipt at the International Bureau: 05 January 2005 (05.01.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland  
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record.**

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

** BLACK BORDERS**

- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT OR DRAWING
- BLURRED OR ILLEGIBLE TEXT OR DRAWING
- SKEWED/SLANTED IMAGES
- COLOR OR BLACK AND WHITE PHOTOGRAPHS
- GRAY SCALE DOCUMENTS

** LINES OR MARKS ON ORIGINAL DOCUMENT**

** REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**

OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**